REMARKS

Claims 1-10 are pending in this application. Claims 1, 2 and 4 are amended. Claim 3 is canceled. Claims 6, 8 and 9 were withdrawn by the Examiner as being drawn to a non-elected subject matter.

Claim 1 is amended to recite "A reversibly immortalized mammalian liver cell line or a passage cell line thereof, containing an immortalizing gene interposed between a pair of site-specific recombination sequences and a suicide gene in the outside of the pair of site-specific recombination sequences, wherein the suicide gene can exhibit its function after excision of the pair of site-specific recombination sequences, wherein the liver cell line or a passage cell line thereof does not contain a promoter derived from virus." Support for the amendment of can be found throughout the present specification as originally filed.

Claim 2 is amended to recite "The reversibly immortalized mammalian liver cell line or the passage cell line thereof of Claim 1, wherein the liver cell line or the passage cell line thereof comprises human liver cells." Support for these amendments can be found throughout the present specification as originally filed.

Claim 4 is amended to remove the parenthetical portion of the claim.

The amendments to the claims are made solely for advancing prosecution. Applicants, by amending or cancelling any claims herein, make no admission as to the validity of any objection and/or rejection made by the Examiner. Applicants reserve the right to reassert the original claim scope of any claim amended herein, in a continuing application.

No new matter is introduced to this application within the meaning of 35 USC §132.

In view of the following, further and favorable consideration is respectfully requested.

I. Election/Restriction

The Examiner recognizes claims 9 and 10, introduced in the Preliminary Amendment filed September 28, 2006, were not included in the Restriction Requirement. The Examiner notes claim 9 should have been included in Group II of the Restriction Requirement and claim 10 should have been included in Group I.

Further, the Examiner acknowledges the election of Group I drawn to claims 1-5, 7 and 10. The Examiner states that the election was treated as an election without traverse because Applicant did not distinctly and specifically point out the supposed errors in the restriction requirement. Applicants respectfully submit the Examiner incorrectly characterized Applicants Response filed October 29, 2009. Applicants direct the Examiner's attention to page 3 of the Response where Applicants explain no serious burden exists to search and examine the entire application. Applicants maintain the election was made with traverse and a proper explanation of the errors of the Restriction Requirement was distinctly and specifically set forth in the Response filed October 29, 2009. Accordingly, the restriction is improper, and the claims should all be prosecuted together in the present application.

II. Rejection of Claims 32 and 33 under 35 U.S.C. §112, 2nd paragraph

Claims 2-5, 7 and 10 are rejected under 35 U.S.C. §112, 2nd paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

With regard to claim 2, the Examiner asserts that the phrase "wherein said mammalian is human" is grammatically incorrect. Claim 2 is amended to recite "wherein the liver cell line or the passage cell line thereof comprises human liver cells." Applicants respectfully submit amended claim 2 is clear and definite and request the Examiner reconsider and withdraw this rejection.

With regard to claim 3, the Examiner asserts the claim is "indefinite because it does not clearly set forth the structure of the promoter or clearly indicate from [where] the promoter cannot come." Applicants canceled claim 3 and incorporated the limitations of claim 3 into claim 1. Claim 1 recites "A reversibly immortalized mammalian liver cell line or a passage cell line thereof, containing an immortalizing gene interposed between a pair of site-specific recombination sequences and a suicide gene in the outside of the pair of site-specific recombination sequences, wherein the suicide gene can exhibit its function after excision of the pair of site-specific recombination sequences, ***wherein the liver cell line or a passage cell line thereof does not contain a promoter derived from virus***." Claim 1 clearly recites the liver cell line or a passage cell line thereof does not contain a promoter derived from virus.

Applicants believe that a person of ordinary skill in the art would clearly understand what a promoter derived from virus means in view of the technical common knowledge at the time the present application was filed. At page 59, lines 1 to 3 of "Animal cell cultures technique and substance production", September 2002, CMC Publishing Co., Ltd. attached herein to this Response as Appendix A, along with an English translation of the relevant section, the process of the single-stranded RNA virus being converted to double stranded DNA by the action of reverse transcriptase encoded by viral *pol* gene is described as

follows:

> In this process, viral DNA has large repeat sequence called "LTR (long terminal repeat)" at each end. An activity as promoter and information for transcriptional termination LTR exist on LTR."

In other words, the reference discloses (a) LTR is generated at the two ends of DNA in the process of preparing a viral vector, and (b) that such LTR have strong promoter activity. Therefore, it is clear that the phrase "a promoter derived from virus" includes viral LTR corresponding to a promoter. Accordingly, Applicants submit the phrase "a promoter derived from virus" is clear and definite to one of ordinary skill in the art at the time the invention was made.

Applicants respectfully submit amended claim 1, which incorporates the limitations of claim 3, is clear and definite and request the Examiner reconsider and withdraw this rejection.

With regard to claim 4, the Examiner asserted the parenthetical phrase of the claim is unclear because it cannot be determined if the parenthetical is part of the claim or not. Applicants amended claim 4 to remove the parenthetical phrase. Applicants respectfully submit amended claim 4 is clear and definite and request the Examiner reconsider and withdraw this rejection.

With regard to claim 5, the Examiner asserted claim 5 is indefinite because the Examiner could not determine how the cell of claim 5 is structurally or functionally different than the cell of claim 1. The cell of claim 1 contains the immortalizing gene. In contrast, the immortalizing gene is excised from the cell of claim 5. Applicants submit the cell of claim 5 has the same function as the cell of claim 1 except for the function relating to proliferation. The functional difference was observed in Test Examples 1 and 3 and

depicted in Figures 6a, 6B and 10. Applicants respectfully submit claim 5 and dependent claim 10 are clear and definite and request the Examiner reconsider and withdraw this rejection.

Accordingly, Applicants respectfully submit that the present claims are clear and definite and respectfully request that the Examiner reconsider and withdraw this rejection.

III. Rejection of Claims 1-5, 7 and 10 under 35 U.S.C. §102(b)

The Examiner asserts that claims 2-5, 7 and 10 are anticipated by Westerman (PNAS, Aug. 1996, Vol. 93, pg 8971-8976), Salmon (Molecular Therapy, Oct. 2000, Vol. 2, No.4, pg 404-414), Kobayashi (Science, Feb. 18,2000, Vol. 287, pg 1258-1262), Kobayashi (Human Cell., March 2000, Vol. 13, No.1, pg 7-13), Kobayashi (Saisei Iryo, Nov. 2002, Vol. 1, No.2, pg 23-28) and Kobayashi (Cell Technology, June 2000, Vol. 19, No.6, pg 864-868). The Examiner asserts all of the references teach a reversibly immortalized human liver cell line containing an immortalizing gene between a pair of site-specific recombination sequences, and a suicide gene in the outside of the pair of site-specific recombination sequences, wherein the suicide gene can exhibit its function after excision of the pair of site-specific recombination sequences.

Applicants respectfully traverse this rejection. The test for anticipation is whether each and every element as set forth is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); MPEP §2131. The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989); MPEP §2131. The elements must also be arranged as

required by the claim. *In re Bond*, 15 USPQ2d 1566 (Fed. Cir. 1990).

In the present application, independent claim 1, as amended, recites "A reversibly immortalized mammalian liver cell line or a passage cell line thereof, containing an immortalizing gene interposed between a pair of site-specific recombination sequences and a suicide gene in the outside of the pair of site-specific recombination sequences, wherein the suicide gene can exhibit its function after excision of the pair of site-specific recombination sequences, wherein the liver cell line or a passage cell line thereof does not contain a promoter derived from virus." Specifically, the liver cell line or a passage cell line thereof ***does not contain a promoter derived from virus***.

Applicants note that the specification as originally filed is amended to remove CMV promoter as a possible non-viral promoter. The CMV promoter is derived from cytomegalovirus and as such cannot be considered a ***non-viral*** promoter. By amending the specification, Applicants are correcting this improper description of the CMV promoter.

Applicants respectfully submit the instant claims are distinguishable from the teachings of all the cited references. All of the promoters described in the cited references are ***promoters derived from virus***, such as a CMV promoter and viral LTR. As mentioned above in section II, viral LTR is included in a promoter derived from a virus.

In contrast, the presently pending claims recite the liver cell line or the passage cell line thereof ***does not contain a promoter derived from virus***. Therefore, the cited references, which all teach a promoter derived from a virus, do not teach each and every element of the presently pending claims and do not anticipate the presently pending claims.

For at least the foregoing reasons, the cited references do not anticipate presently pending claims 1-5, 7 and 10 within the meaning of 35 USC § 102(b). Accordingly, Applicants respectfully request the Examiner to reconsider and withdraw this rejection.

CONCLUSION

In view of the foregoing, Applicants submit the application is in condition for immediate allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to contact the undersigned attorney if it is believed such contact will expedite the prosecution of the application.

In the event this paper is not timely filed, Applicants petition for an appropriate extension of time. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 14-0112.

Respectfully submitted,

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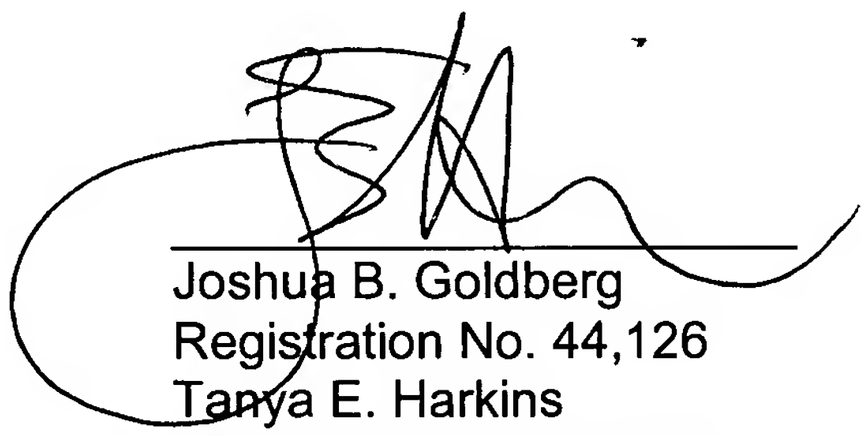

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APPENDIX A

2.2.6　宿主・ベクター系

(1)　SV40－サル細胞系

SV40ゲノムは，5243塩基からなる2本鎖閉環状DNAで，遺伝子構造やその発現が，よく解析されている。複製起点（*ori*）をはさんで，初期（DNA合成前）遺伝子と後期（DNA合成開始後）遺伝子の2領域がある。前者からは，large T抗原とsmall T抗原が，後者からは，VP1，VP2やVP3が合成される。ウイルスの自己複製，細胞の形質転換には，large T抗原が必要である。

SV40をベクターとして用いる場合，アフリカミドリザル腎細胞由来のCV-1が，宿主としてよく用いられている。組換え遺伝子を作製する際には，(i)後期遺伝子領域に目的遺伝子を挿入する場合と(ii)初期遺伝子領域に挿入する場合があるが，SV40感染細胞では，後期プロモーター活性が強いために，前者を用いることが多い。HamerとLeder[28]は，β^{maj}グロビン遺伝子をSV40後期領域に挿入した実験より，安定な細胞質mRNAを得るためには，組換え遺伝子が，少なくとも1つの，欠損のない（intact）splice junctionをもたなければならないことを示した。後期置換ベクター系にて，H-*ras*[29]，ラットプロインシュリン[30]そしてHBsAg[31]などの発現が成功している。

SV40が宿主細胞で自己増殖するには，前述のように，large T抗原の存在が必須である。したがって，large T抗原がコードされている初期領域の置換はほとんどなされなかった。しかし，Gluzman[32]がCV-1を*ori*欠損のSV40で形質転換したCOS細胞を樹立したために，この系が多用され始めた。ただ，この系では，組換え遺伝子の増幅・転写が一過性であり，細胞はやがて死滅する。インフルエンザウイルス血球凝集素遺伝子やヒトγ型インターフェロン[33]の発現が成功しているが，その生産性は，後期置換ベクター系にくらべて低い。

(2)　アデノウイルス－ヒト細胞系

アデノウイルス（AdV）は，直鎖状2本鎖のDNAであり，約35kb塩基からなる大型のものである。DNAの5′末端には，分子量55,000のタンパク質が結合しているが，このタンパク質はDNA複製におけるプライマー機能を有し，かつ感染効率にも関与している。AdV感染ヒト細胞におけるAdV遺伝子の発現は，SV40に似ているが，かなり複雑である。AdVは5つの初期転写単位（*E1A*, *E1B*, *E2*, *E3*, *E4*）を有し，DNA合成後には，16.6 mp（map position）にある後期プロモーター（major late promoter, MLP）より，十数種の後期mRNAが転写される。AdVのMLPは非常に強力であることが知られている。この系に関する詳細は，小田の総説[5]にゆずりたい。

(3)　レトロウイルスベクター

レトロウイルスは，1本鎖RNAウイルスであるが，感染細胞内で，ウイルスの*pol*遺伝子に

コードされている逆転写酵素により，2本鎖DNA（プロウイルス）に変換される。この過程で，ウイルスDNAは，両端にLTR（long terminal repeat）と呼ばれる大きな反復配列をもつ。LTRには，プロモーター活性や転写終結の情報が存在する。また，細胞DNAへの組込みにもLTRが関与する。

レトロウイルスベクター系は，以下に記するいくつかの特徴を有する。

(i)広い宿主域をもつ，

(ii)感染細胞内でウイルスDNAが安定である，

(iii)感染効率が高く，ウイルス感染により細胞が死滅しない，

(iv)genomicあるいはsubgenomic DNAを挿入した場合に，スプライシングが起こる，

(v)細胞に導入できるゲノムの大きさが8～9kbと大きい。

この系において，c-*mos*[34]，v-*ras*H[35]やラット成長ホルモン[36]の発現が報告されている。この系に関しては，文献37)を参照していただきたい。

(4) エピゾームベクター

SV40，AdVやレトロウイルスの系では，ベクターは宿主細胞DNAに組込まれるが，細胞DNAに組込まれることなく，プラスミドレプリコンとして増殖するものがある。

①ウシパピローマウイルス（BPV）－マウス細胞系

パピローマウイルスは，SV40やポリオーマウイルスなどとともに，パポーバウイルス群に属し，宿主に乳頭腫（良性のイボ）を誘発するウイルスである。パピローマウイルスの中のひとつ，BPVは，ハムスターやマウスに繊維芽細胞腫をおこし，培養細胞を形質転換し，フォーカス（focus）形成をひきおこすこと，これらの細胞内ではウイルスゲノムがエピゾームとして存在すること，が判明した。こうした事実から，この系に対して，細菌におけるプラスミドと同じような，ベクターとしての機能が期待された。BPVゲノムがエピゾームとして存在する細胞株として，マウスC127，NIH3T3，ラットFR3T3がある。これらの細胞では，多くの組換え遺伝子が数十コピー/細胞のエピゾームとして存在することが示されているが，例外として，HSV-*tk*遺伝子，マウス*dhfr*遺伝子[38]，組織適合性抗原HLA-H鎖遺伝子[39]，等が知られている。Lowryら[40]は，BPVゲノムの69%の長さの*Bam*HI-*Hin*dⅢ断片が，マウス細胞を形質転換するのに十分であることを示した。この系での物質生産の例を紹介したい。

[a]HBsAgの発現[41]

HBVのsubgenomic断片を，細菌プラススミドpML2dと共にBPV（100%）に組換え，C127細胞に導入し，フォーカス形成細胞をクローン化した。大部分のクローンがHBsAgを培地に分泌しており，糖付加が起こっていた。クローン内で，組換え遺伝子はエピゾームとして存在したが，分子内再配列（rearrangement）を受けていた。

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Page 58, line 2 from the bottom to page 59, line 3

(3) Retrovirus vector

Retrovirus is single-stranded RNA virus, but in infected cell it is converted to double-stranded DNA (provirus) by the action of reverse transcriptase encoded by viral *pol* gene. In this process, viral DNA has large repeat sequence called "LTR (long terminal repeat)" at each end. An activity as promoter and information for transcriptional termination LTR exist on LTR."

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